

CLAIMS

What is claimed is:

1. A recombinant adenovirus comprising an adenovirus that encodes one or more AAV REP78/68 polypeptides, wherein the one or more AAV REP78/68 polypeptides is inducibly expressed.
2. The recombinant adenovirus of claim 1, wherein the recombinant adenovirus encodes one or more REP78/68 polypeptides following serial passage of the recombinant adenovirus.
3. The recombinant adenovirus of claim 1, wherein the one or more AAV REP78/68 polypeptide comprises an AAV-2 REP78/68 polypeptide.
4. The recombinant adenovirus of claim 1, wherein an AAV REP78/68 polypeptide of the one or more AAV REP78/68 polypeptides comprises a REP78 polypeptide, a REP68 polypeptide, or a combination thereof.
5. The recombinant adenovirus of claim 4, wherein the REP78 polypeptide comprises:
 - (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:2; or
 - (b) a polypeptide substantially identical to SEQ ID NO:2.
6. The recombinant adenovirus of claim 4, wherein the REP68 polypeptide comprises:
 - (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:4; or
 - (b) a polypeptide substantially identical to SEQ ID NO:4.
7. The recombinant adenovirus of claim 1, further comprising:
 - (a) a nucleic acid molecule encoding the one or more AAV REP78/68 polypeptides; and
 - (b) an inducible promoter, wherein the inducible promoter is operatively linked to the nucleic acid molecule encoding the one or more AAV REP78/68 polypeptides.

8. The recombinant adenovirus of claim 7, wherein the nucleic acid molecule encoding the one or more AAV REP78/68 polypeptides comprises:

- (a) a nucleotide sequence of SEQ ID NO:1 or 3;
- 5 (b) a nucleotide sequence substantially similar to SEQ ID NO:1 or 3; or
- (c) a combination thereof.

9. The recombinant adenovirus of claim 7, wherein the inducible promoter comprises a chemically-inducible promoter or a heat-inducible promoter.

10. The recombinant adenovirus of claim 9, wherein the chemically-inducible promoter comprises a tetracycline-inducible promoter.

11. The recombinant adenovirus of claim 9, wherein the heat-inducible promoter comprises a *hsp70* promoter.

15 12. The recombinant adenovirus of claim 12, wherein the *hsp70* promoter comprises:

- (a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:14; or
- (b) a nucleic acid molecule substantially identical to SEQ ID 20 NO:14.

13. The recombinant adenovirus of claim 1, further comprising one or more AAV REP52/40 polypeptides, wherein the one or more AAV REP52/40 polypeptides is constitutively expressed.

25 14. The recombinant adenovirus of claim 13, wherein the one or more AAV REP52/40 polypeptides comprise an AAV-2 REP52/40 polypeptide.

15. The recombinant adenovirus of claim 10, wherein the one or more REP52/40 polypeptides comprises a REP52 polypeptide, a REP40 polypeptide, or a combination thereof.

30 16. The recombinant adenovirus of claim 15, wherein the REP52 polypeptide comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:6; or
- (b) a polypeptide substantially identical to SEQ ID NO:6.

17. The recombinant adenovirus of claim 15, wherein the REP40

5 polypeptide comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:7; or
- (b) a polypeptide substantially identical to SEQ ID NO:7.

18. The recombinant adenovirus of claim 13, further comprising:

10 (a) a nucleic acid molecule encoding one or more AAV REP52/40 polypeptides; and

- (b) a constitutive promoter, wherein the constitutive promoter is operatively linked to the nucleic acid molecule encoding an AAV REP52/40 polypeptide.

15 19. The recombinant adenovirus of claim 18, wherein the nucleic acid molecule encoding one or more AAV REP52/40 polypeptide comprises:

- (a) a nucleotide sequence of SEQ ID NO:5; or
- (b) a nucleotide sequence substantially similar to SEQ ID NO:5.

20. The recombinant adenovirus of claim 18, wherein the

20 constitutive promoter comprises an AAV-2 p19 promoter.

21. The recombinant adenovirus of claim 1, further comprising one or more viral capsid polypeptides.

22. The recombinant adenovirus of claim 21, wherein the one or more viral capsid polypeptides comprises a chimeric viral capsid.

25 23. The recombinant adenovirus of claim 21, wherein the one or more viral capsid polypeptides comprise one or more AAV-2 CAP polypeptides.

24. The recombinant adenovirus of claim 21, wherein the one or more viral capsid polypeptides comprise an AAV VP1 polypeptide, an AAV

30 VP2 polypeptide, an AAV VP3 polypeptide, or a combination thereof.

25. The recombinant adenovirus of claim 24, wherein the VP1 polypeptide comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:9; or
- (b) a polypeptide substantially identical to SEQ ID NO:9.

26. The recombinant adenovirus of claim 24, wherein the VP2 polypeptide comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:10; or
- (b) a polypeptide substantially identical to SEQ ID NO:10.

27. The recombinant adenovirus of claim 24, wherein the VP3 polypeptide comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:11; or
- (b) a polypeptide substantially identical to SEQ ID NO:11.

28. The recombinant adenovirus of claim 21, further comprising a nucleic acid molecule, wherein the nucleic acid molecule encodes the one or more viral capsid polypeptides, the nucleic acid molecule comprising:

- (a) a nucleic acid molecule encoding one or more viral capsid polypeptides; and
- (b) a constitutive promoter, wherein the constitutive promoter is operatively linked to the nucleic acid molecule encoding the one or more viral capsid polypeptides.

29. The recombinant adenovirus of claim 28, wherein the nucleic acid molecule encoding the one or more viral capsid polypeptides comprises:

- (a) a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:8; or
- (b) a nucleic acid molecule substantially identical to SEQ ID NO:8.

30. The recombinant adenovirus of claim 28, wherein the constitutive promoter comprises a CMV promoter.

31. A host cell comprising the recombinant adenovirus of claim 1.

32. A complete virus-mediated system for recombinant AAV production comprising:

- (a) a first recombinant adenovirus encoding one or more AAV REP78/68 polypeptides and one or more viral capsid polypeptides;
- (b) a second recombinant adenovirus comprising a gene of interest and AAV inverted terminal repeats, wherein the AAV inverted terminal repeats flank the gene of interest;
- 5 (c) viral helper functions; and
- (d) a host cell comprising the first recombinant adenovirus, the second recombinant adenovirus, and the viral helper functions.

10 33. The system of claim 32, wherein the first recombinant adenovirus encodes one or more REP78/68 polypeptides and one or more viral capsid polypeptides following serial passage of the first recombinant adenovirus.

34. The system of claim 32, wherein the one or more AAV

15 REP78/68 polypeptides comprises an AAV-2 REP78/68 polypeptide.

35. The system of claim 32, wherein an AAV REP78/68 polypeptide of the one or more AAV REP78/68 polypeptides comprises a REP78 polypeptide, a REP68 polypeptide, or a combination thereof.

36. The system of claim 35, wherein the REP78 polypeptide

20 comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:2; or
- (b) a polypeptide substantially identical to SEQ ID NO:2.

37. The system of claim 35, wherein the REP68 polypeptide

25 comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:4; or
- (b) a polypeptide substantially identical to SEQ ID NO:4.

38. The system of claim 32, wherein the first recombinant

30 adenovirus further comprises:

- (a) a nucleic acid molecule encoding the one or more AAV REP78/68 polypeptides; and

(b) an inducible promoter, wherein the inducible promoter is operatively linked to the nucleic acid molecule encoding the one or more AAV REP78/68 polypeptides.

39. The system of claim 38, wherein the nucleic acid molecule 5 encoding the one or more AAV REP78/68 polypeptides comprises:

- (a) a nucleotide sequence of SEQ ID NO:1 or 3;
- (b) a nucleotide sequence substantially similar to SEQ ID NO:1 or 3; or
- (c) a combination thereof.

10 40. The system of claim 38, wherein the inducible promoter comprises a chemically-inducible promoter or a heat-inducible promoter.

41. The system of claim 40, wherein the chemically-inducible promoter comprises a tetracycline-inducible promoter.

42. The system of claim 40, wherein the heat-inducible promoter 15 comprises a *hsp70* promoter.

43. The system of claim 42, wherein the *hsp70* promoter comprises:

- (a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:14; or
- (b) a nucleic acid molecule substantially identical to SEQ ID NO:14.

44. The system of claim 32, wherein the first recombinant adenovirus further comprises one or more AAV REP52/40 polypeptides, wherein the one or more AAV REP52/40 polypeptides is constitutively 25 expressed.

45. The system of claim 44, wherein the one or more AAV REP52/40 polypeptides comprises an AAV-2 REP52/40 polypeptide.

46. The system of claim 44, wherein the one or more REP52/40 polypeptides comprises a REP52 polypeptide, a REP40 polypeptide, or a 30 combination thereof.

47. The system of claim 46, wherein the REP52 polypeptide comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:6; or

(b) a polypeptide substantially identical to SEQ ID NO:6.

48. The system of claim 46, wherein the REP40 polypeptide 5 comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:7; or

(b) a polypeptide substantially identical to SEQ ID NO:7.

49. The system of claim 44, wherein the first recombinant 10 adenovirus further comprises:

- (a) a nucleic acid molecule encoding one or more AAV REP52/40 polypeptides; and

(b) a constitutive promoter, wherein the constitutive promoter is operatively linked to the nucleic acid molecule encoding an 15 AAV REP52/40 polypeptide.

50. The system of claim 49, wherein the nucleic acid molecule encoding the one or more AAV REP52/40 polypeptide comprises:

- (a) a nucleotide sequence of SEQ ID NO:5; or

(b) a nucleotide sequence substantially similar to SEQ ID NO:5.

51. The system of claim 49, wherein the constitutive promoter 20 comprises an AAV-2 p19 promoter.

52. The system of claim 32, wherein the one or more viral capsid polypeptides comprises a chimeric viral capsid.

53. The system of claim 32, wherein the one or more viral capsid 25 polypeptides comprises one or more AAV-2 CAP polypeptides.

54. The system of claim 32, wherein the one or more viral capsid polypeptides comprises an AAV VP1 polypeptide, an AAV VP2 polypeptide, an AAV VP3 polypeptide, or a combination thereof.

55. The system of claim 54, wherein the VP1 polypeptide 30 comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:9; or

(b) a polypeptide substantially identical to SEQ ID NO:9.

56. The system of claim 54, wherein the VP2 polypeptide comprises:

(a) a polypeptide comprising an amino acid sequence of SEQ ID NO:10; or

(b) a polypeptide substantially identical to SEQ ID NO:10.

57. The system of claim 54, wherein the VP3 polypeptide comprises:

(a) a polypeptide comprising an amino acid sequence of SEQ ID NO:11; or

(b) a polypeptide substantially identical to SEQ ID NO:11.

58. The system of claim 32, wherein the first recombinant adenovirus further comprises a nucleic acid molecule, wherein the nucleic acid molecule encodes the one or more AAV viral capsid polypeptides, the nucleic acid molecule comprising:

(a) a nucleic acid molecule encoding one or more viral capsid polypeptides; and

(b) a constitutive promoter, wherein the constitutive promoter is operatively linked to the nucleic acid molecule encoding the one or more viral capsid polypeptides.

59. The system of claim 58, wherein the nucleic acid molecule encoding one or more viral capsid polypeptides comprises:

(a) a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:8; or

(b) a nucleic acid molecule substantially identical to SEQ ID NO:8.

60. The system of claim 58, wherein the constitutive promoter comprises a CMV promoter.

61. The system of claim 32, wherein the gene of interest comprises a reporter gene, a gene encoding a therapeutic polypeptide, a gene encoding a therapeutic oligonucleotide, or a combination thereof.

62. The system of claim 32, wherein the AAV inverted terminal repeats comprise inverted terminal repeats of a same AAV serotype as the one or more AAV REP78/68 polypeptides.

63. The system of claim 32, wherein the AAV inverted terminal repeats comprise AAV-2 inverted terminal repeats and the one or more AAV REP78/68 polypeptides comprise an AAV-2 REP78/68 polypeptide.

64. The system of claim 32, wherein the viral helper functions comprise a helper virus.

65. The system of claim 32, wherein the helper virus comprises a wild type adenovirus.

66. The system of claim 32, wherein the host cell comprises a packaging cell free of AAV REP78/68 polypeptide prior to infection with the recombinant adenovirus encoding one or more AAV REP78/68 polypeptides and one or more viral capsid polypeptides.

67. The system of claim 66, wherein the host cell comprises a 293 human embryonic kidney cell.

68. The system of claim 66, wherein the host cell comprises an E1-deficient cell.

69. The system of claim 67, wherein the host cell comprises a HeLa cell.

70. A method for producing a recombinant AAV comprising:

(a) providing to a host cell:

(i) a first recombinant adenovirus encoding one or more AAV REP78/68 polypeptides and one or more viral capsid polypeptides;

(ii) a second recombinant adenovirus comprising a gene of interest and AAV inverted terminal repeats, wherein the AAV inverted terminal repeats flank the gene of interest; and

(iii) viral helper functions; and

(b) culturing the host cell, whereby a recombinant AAV is produced.

71. The method of claim 70, wherein the providing comprises infecting a host cell with:

- (a) a recombinant adenovirus encoding one or more AAV REP78/68 polypeptides and one or more viral capsid polypeptides;
- (b) a recombinant adenovirus comprising a gene of interest and AAV inverted terminal repeats, wherein the AAV inverted terminal repeats flank the gene of interest; and
- (c) a helper virus.

10 72. The method of claim 70, wherein the host cell comprises a host cell free of AAV REP78/68 polypeptide prior to infection with the recombinant adenovirus encoding one or more AAV REP78/68 polypeptides and one or more viral capsid polypeptides.

15 73. The method of claim 72, wherein the host cell comprises a 293 human embryonic kidney cell.

74. The method of claim 72, wherein the host cell comprises an E1-deficient cell.

75. The method of claim 74, wherein the host cell comprises a HeLa cell.

20 76. The method of claim 70, wherein the first recombinant adenovirus encodes one or more REP78/68 polypeptides and one or more viral capsid polypeptides following serial passage of the first recombinant adenovirus.

77. The method of claim 70, wherein the one or more AAV 25 REP78/68 polypeptides comprises an AAV-2 REP78/68 polypeptide.

78. The method of claim 70, wherein an AAV REP78/68 polypeptide of the one or more AAV REP78/68 polypeptides comprises a REP78 polypeptide, a REP68 polypeptide, or a combination thereof.

79. The method of claim 78, wherein the REP78 polypeptide 30 comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:2; or

(b) a polypeptide substantially identical to SEQ ID NO:2.

80. The method of claim 78, wherein the REP68 polypeptide comprises:

5 (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:4; or

(b) a polypeptide substantially identical to SEQ ID NO:4.

81. The method of claim 70, further comprising:

10 (a) a nucleic acid molecule encoding the one or more AAV REP78/68 polypeptides; and

(b) an inducible promoter, wherein the inducible promoter is operatively linked to the nucleic acid molecule encoding the one or more AAV REP78/68 polypeptides.

82. The method of claim 81, wherein the nucleic acid molecule encoding the one or more AAV REP78/68 polypeptides comprises:

15 (a) a nucleotide sequence of SEQ ID NO:1 or 3;

(b) a nucleotide sequence substantially similar to SEQ ID NO:1 or 3; or

(c) a combination thereof.

83. The system of claim 81, wherein the inducible promoter

20 comprises a chemically-inducible promoter or a heat-inducible promoter.

84. The method of claim 83, wherein the chemically-inducible promoter comprises a tetracycline-inducible promoter.

85. The method of claim 84, further comprising:

25 (a) providing to the host cell a tetracycline or a tetracycline analog; and

(b) providing to the host cell a reverse tet-responsive transcriptional activator polypeptide, whereby a REP78/68 polypeptide is produced.

86. The method of claim 85, wherein the providing the reverse tet-

30 responsive transcriptional activator polypeptide comprises infecting the host cell with a recombinant adenovirus encoding the reverse tet-responsive transcriptional activator polypeptide.

87. The method of claim 83, wherein the heat-inducible promoter comprises a *hsp70* promoter.

88. The method of claim 87, wherein the *hsp70* promoter comprises:

- 5 (a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:14; or
- (b) a nucleic acid molecule substantially identical to SEQ ID NO:14.

89. The method of claim 83, wherein the inducible promoter is a heat-inducible promoter, and further comprising heating the host cell to induce expression of the one or more REP78/68 polypeptides.

90. The method of claim 70, wherein the first recombinant vector further comprises one or more AAV REP52/40 polypeptides, wherein the one or more AAV REP52/40 polypeptides is constitutively expressed.

15 91. The method of claim 90, wherein the one or more AAV REP52/40 polypeptides comprises an AAV-2 REP52/40 polypeptide.

92. The method of claim 90, wherein the one or more REP52/40 polypeptides comprises a REP52 polypeptide, a REP40 polypeptide, or a combination thereof.

20 93. The method of claim 92, wherein the REP52 polypeptide comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:6; or
- (b) polypeptide substantially identical to SEQ ID NO:6.

25 94. The method of claim 92, wherein the REP40 polypeptide comprises:

- (a) a polypeptide comprising an amino acid sequence of SEQ ID NO:7; or
- (b) a polypeptide substantially identical to SEQ ID NO:7.

30 95. The method of claim 70, wherein the first recombinant vector further comprises:

(a) a nucleic acid molecule encoding one or more AAV REP52/40 polypeptides; and
(b) a constitutive promoter, wherein the constitutive promoter is operatively linked to the nucleic acid molecule encoding an
5 AAV REP52/40 polypeptide.

96. The method of claim 95, wherein the nucleic acid molecule encoding the one or more AAV REP52/40 polypeptide comprises:

(a) a nucleotide sequence of SEQ ID NO:5; or
(b) a nucleotide sequence substantially similar to SEQ ID NO:5.
10 97. The method of claim 95, wherein the constitutive promoter comprises an AAV-2 p19 promoter.

98. The method of claim 70, wherein one or more viral capsid polypeptides comprises a chimeric viral capsid.

99. The method of claim 70, wherein the one or more viral capsid 15 polypeptides comprises one or more AAV-2 CAP polypeptides.

100. The method of claim 70, wherein the one or more viral capsid polypeptides comprise an AAV VP1 polypeptide, an AAV VP2 polypeptide, an AAV VP3 polypeptide, or a combination thereof.

101. The method of claim 100, wherein the VP1 polypeptide 20 comprises:

(a) a polypeptide comprising an amino acid sequence of SEQ ID NO:9; or
(b) a polypeptide substantially identical to SEQ ID NO:9.

102. The method of claim 100, wherein the VP2 polypeptide 25 comprises:

(a) a polypeptide comprising an amino acid sequence of SEQ ID NO:10; or
(b) a polypeptide substantially identical to SEQ ID NO:10.

103. The method of claim 100, wherein the VP3 polypeptide 30 comprises:

(a) a polypeptide comprising an amino acid sequence of SEQ ID NO:11; or

(b) a polypeptide substantially identical to SEQ ID NO:11.

104. The method of claim 70, wherein the first recombinant vector further comprises a nucleic acid molecule, wherein the nucleic acid molecule encodes the one or more viral capsid polypeptides, the nucleic acid 5 molecule comprising:

- (a) a nucleic acid molecule encoding one or more viral capsid polypeptides; and
- (b) a constitutive promoter, wherein the constitutive promoter is operatively linked to the nucleic acid molecule encoding the one or more viral capsid polypeptides.

10 105. The method of claim 104, wherein the nucleic acid molecule encoding one or more viral capsid polypeptides comprises:

- (a) a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:8; or
- 15 (b) a nucleic acid molecule substantially identical to SEQ ID NO:8.

106. The method of claim 104, wherein the constitutive promoter comprises a CMV promoter.

107. The method of claim 70, wherein the gene of interest comprises a reporter gene, a gene encoding a therapeutic polypeptide, a 20 gene encoding a therapeutic oligonucleotide, or a combination thereof.

108. The method of claim 70, wherein the AAV inverted terminal repeats comprise inverted terminal repeats of a same AAV serotype as the one or more AAV REP78/68 polypeptides.

109. The method of claim 70, wherein the AAV inverted terminal 25 repeats comprise AAV-2 inverted terminal repeats and the one or more AAV REP78/68 polypeptides comprise an AAV-2 REP78/68 polypeptide.

110. The method of claim 70, wherein the viral helper functions comprise a helper virus.

111. The method of claim 110, wherein the helper virus comprises a 30 wild type adenovirus.

112. The method of claim 70, further comprising purifying the recombinant AAV.

113. The method of claim 70, further comprising preparing a host cell lysate, heating the lysate to a sufficient temperature to inactivate adenovirus, and recovering the recombinant AAV.

114. The method of claim 70, wherein the recombinant AAV is 5 substantially free of infectious adenovirus.

115. The method of claim 70, wherein the recombinant AAV comprises at least about 200 infectious units per host cell to about 600 infectious units per host cell.

116. The method of claim 115, wherein the rAAV comprises at least 10 about 200 infectious units per host cell to about 600 infectious units per host cell.

117. The method of claim 116, wherein the rAAV comprises at least about 400 infectious units per host cell to about 600 infectious units per host cell.

118. The method of claim 70, wherein the rAAV comprises at least 15 about 10^5 virus particles per cell.

119. The method of claim 112, wherein the rAAV comprises at least about 10^6 virus particles per cell.